Environ. Sci. Technol. **2005**, *39,* 4505−4512

Cesium Desorption from Illite as Affected by Exudates from Rhizosphere Bacteria

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Biogeochemical processes in the rhizosphere can significantly alter interactions between contaminants and soil minerals. In this study, several strains of bacteria that exude aluminum (Al)-chelating compounds were isolated from the rhizosphere of crested wheatgrass (*Agropyron desertorum*) collected from the Idaho National Laboratory (INL). We examined the effects of exudates from bacteria in the genera *Bacillus*, *Ralstonia*, and *Enterobacter* on cesium (Cs) desorption from illite. Exudates from these strains of bacteria significantly enhanced Cs desorption from illite. In addition, Cs desorption increased with increasing *Bacillus* exudate concentrations. Cesium desorption from illite as a function of both exudate type and concentration was positively correlated with Al dissolution, suggesting that the Al-complexing ability of the exudates played an important role in enhancing Cs desorption. The density of frayed edge sites (FES) on illite increased as a result of treatment with bacterial exudates, while the Cs/K selectivity of FES decreased. These results suggest that exudates from bacteria isolated from the rhizosphere can enhance Cs desorption from frayed edges of illite and, therefore, can alter Cs availability in micaceous soils.

Introduction

Cesium (Cs) is the most frequently encountered radionuclide contaminant at current and former Department of Energy sites in the U.S. (*1*) and, as such, is a key contaminant to be considered in ecosystem restoration strategies. Cesium is commonly found in shallow soil systems contaminated as a result of nuclear weapons testing, nuclear reactor accidents, and past waste disposal practices at nuclear facilities. Phytoremediation has been suggested as a cost-effective strategy for the remediation of soils contaminated with radionuclides and heavy metals. Cesium may be particularly amenable to phytoremediation because Cs has a similar ionic potential to the plant nutrient potassium (K) and has been observed to exhibit many similarities to K with respect to plant uptake (*2*, *3*). Knowledge of the nature and reversibility of Cs sorption to the soil solid phase is essential to evaluate the potential of phytoremediation for Cs removal from soil and to develop contaminant removal technologies.

Phyllosilicate minerals comprise the majority of the reactive solid phase in many low organic matter temperate soils. Cesium interactions with phyllosilicate mineral surfaces are relatively strong due to the formation of inner sphere complexes. Cesium bound to external planar surface sites can generally be exchanged by other cations, whereas that bound in collapsed (dehydrated) interlayer sites or at frayed edges of a collapsed interlayer is more strongly sorbed and difficult to remove via ion exchange (*4*). The availability of interlayer sites for cation exchange depends on interlayer spacing, which is influenced by the degree of hydration of the ions present in the interlayer. Cations with low hydration energy such as Cs, K, rubidium (Rb), and ammonium (NH_4) can shed their hydration shell and enter clay interlayers. Dehydration of these ions permits a close approach to the tetrahedral silicate layers and formation of polar bonds with structural oxygen atoms. Cesium bound to interlayer sites is not readily exchanged by other cations and is generally considered fixed (*4*).

A strong surface association between Cs and the soil solid phase occurs at frayed edge sites (FES) of micaceous phyllosilicate minerals. Plant root and microbial exudates may enhance Cs bioavailability in the rhizosphere by accelerating weathering at frayed edges of phyllosilicate minerals and releasing Cs sorbed to frayed edge and interlayer sites, making the ion available for uptake. The FES of illite, a secondary clay mineral similar to muscovite mica, have a particularly high affinity for Cs (*5*, *6*). Characterization of Cs desorption from illite will provide insight regarding Cs dynamics in contaminated rhizosphere soils, in particular, the potential applicability of phytoremediation for the removal of Cs from contaminated soils.

A selectivity coefficient describing the exchange properties of regular (planar) exchange sites or FES with respect to Cs can be determined by comparing the retention of Cs in relation to a competing ion, such as K or NH_4. Cation exchange on accessible sites of expandable phyllosilicate minerals or soil organic matter occurs easily with relatively small differences in selectivity among alkali and alkaline earth metals. Exchange on sites at collapsible interlayers such as those found in micas and vermiculite, on the other hand, show high selectivity for large alkali metals (K, NH_4, Cs) over their smaller counterparts (Na, Li) or alkaline earth metals (Ca, Mg). The less negative hydration energy of the large alkali metals allows dehydration and collapse of the interlayer or sites near the edges of collapsed interlayers of micas (FES). As a result, FES can exhibit very high selectivities for large alkali metals ($Cs > NH_4 > K$) relative to other cations (*4*, *6*−*9*). In this work, we operationally define FES as those sites that will retain sorbed Cs and K against exchange with a large excess of Ca but not against exchange with a large excess of NH_4 (*8*).

The zone of soil influenced by plant roots, the soil rhizosphere, is chemically, biologically, and mineralogically distinct from bulk soil (*7*). The soil solution composition and pH in the rhizosphere can be appreciably different from that of the surrounding bulk soil due to the presence of plant and microbial communities and associated organic exudates.

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10.1021/es048809p CCC: $30.25
Published on Web 05/14/2005

Many studies have documented the significance of exudates from plant roots and rhizosphere microorganisms to phyllosilicate mineral weathering processes in the soil environment (*10*−*17*). We recently investigated the effects of oxalate, a surrogate plant root exudate, on Cs interactions with illite (*11*). Here, we examine Cs desorption from illite in the presence of exudates from bacteria found in the rhizosphere of crested wheatgrass (*Agropyron desertorum*) and analyze the potential contribution of bacteria to illite weathering in the crested wheatgrass rhizosphere.

The Idaho National Laboratory (INL) in southeastern Idaho was established as the National Reactor Testing Station in 1949 and was once the site of the world's largest concentration of nuclear reactors. A large portion of the area that is now the INL was used as a gunnery and bombing range by the U.S. Navy and U.S. Army during World War II. Radionuclide and metal contamination of soils at the INL is limited to areas of localized accidents or spills and where releases or disposal have occurred (*18*). Recent surveys at the INL have shown levels of ^{137}Cs in the top 30 cm of soil ranging from nondetectable to more than 0.1 μCi kg^{-1} (3700 Bq kg^{-1} or 0.15 μmol kg^{-1} ^{137}Cs), with a total mean nonradioactive Cs concentration of approximately 20−30 μmol kg^{-1} (*19*). Soils from a noncontaminated area of the INL exhibited a median ^{137}Cs activity of 1.3 pCi/g (*19*).

Exudates from bacteria isolated from the rhizosphere of crested wheatgrass were selected for use in this study after field surveys of ^{137}Cs levels in three plant species representing the dominant vegetation at the INL—rabbitbrush (*Chrysothammus viscidiflorus*), sagebrush (*Artemesia tridentata*), and crested wheatgrass—indicated that approximately 94% of ^{137}Cs accumulation in aboveground biomass was associated with crested wheatgrass (*19*). Crested wheatgrass plants from a contaminated site at the INL exhibited median radioactivities of 0.93 and 100 pCi/g dry biomass for shoots and roots, respectively (*19*). Within a noncontaminated control site, shoots and roots of crested wheatgrass exhibited mean radioactivities of 0.22 and 0.94 pCi/g dry biomass, respectively (*19*).

Materials and Methods

Rhizosphere Soil and Bacteria Characterization. Noncontaminated surface (0−10 cm) soils collected from the SL-1 area in the south-central part of the INL were collected and analyzed using X-ray diffraction (XRD). The major mineral phases identified in the clay (<2 μm) size fraction were illite, smectite, and illite−smectite interlayered minerals. Total K analysis following the method developed by Bernas (*20*) indicated that the clay fraction of the INL soil was comprised of approximately 30% illite. Illite was selected for further experimentation based on evidence that Cs adsorption to the soil solid phase is largely determined by the quantity of illite present (*5*, *12*).

Several strains of bacteria were isolated from the rhizosphere of crested wheatgrass plants obtained from a noncontaminated portion of the SL-1 area of the INL. Individual bacterial strains were identified to genus using 16S rRNA sequencing. Bacterial cells were cultured at 30 °C in a medium containing 8.6 mM NaCl, 18.7 mM NH_4Cl, 0.2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 1.0 mM K_2HPO_4 with glycerol as the carbon source and buffered with PIPES ($C_8H_{18}N_2O_6S_2$, 1,4-piperazinediethanesulfonic acid) to pH 6.3. We used glycerol as a carbon source because of those carbon sources tested glycerol resulted in the most active supernatant solutions in terms of the Al-complexing activity of resulting exudate solutions. The bacteria were cultured aerobically at 30 °C, which was thought to be most appropriate for environmental isolates. Following growth, cells were centrifuged, and the supernatant was filtered to 0.2 μm, then lyophilized and redissolved in one-tenth volume deionized water.

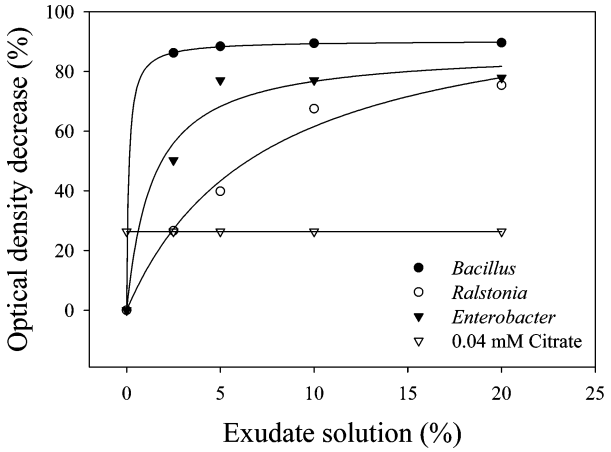

FIGURE 1. Percent decrease in optical density of solution containing Al-pyrocatechol violet as a function of *Bacillus*, *Ralstonia*, and *Enterobacter* exudate concentration.

Bacterial supernatant solutions were assayed for aluminum (Al)-complexing activity using a spectrophotometric Al−pyrocatechol violet (PCV) assay. The Al−PCV assay used here is a modification of the classic chrome azurol S-iron assay for detection of siderophores, developed by Schwyn and Neilands (*21*). In this technique, any Al-complexing component present in a bacterial supernatant solution removes Al from a preformed Al−PCV complex and results in a decrease in optical density of the solutions, monitored at 575 nm. Of those bacteria isolated from the rhizosphere of crested wheatgrass, exudates from bacteria in the genera *Bacillus*, *Ralstonia*, and *Enterobacter* exhibited the greatest Al-complexing activity and were selected for further experimentation. These genera are representative of bacteria that can be found in the soil rhizosphere, although they are not necessarily major genera found in all rhizosphere soils.

For purposes of comparison to Cs desorption in the presence of simulated crested wheatgrass root exudates (*11*), bacterial exudate concentrations for use in these experiments were selected based on the measured Al-binding activity and normalized to approximately equal that of 0.04 mM citric acid. A standard curve relating the citrate concentration to dissociation of the Al−PCV complex was used to determine a citrate equivalent concentration for each bacterial supernatant. On the basis of the assay, 0.2, 1, and 2% (v/v) dilutions of the *Bacillus*, *Ralstonia*, and *Enterobacter* 10X concentrated exudate solutions, respectively, were visually estimated to have Al-binding capabilities equivalent to 0.04 mM citric acid (Figure 1). The formation energies of Al−oxalate and Al−citrate complexes are similar and range from approximately −1122 to −1186 kcal mol^{-1} (*22*). Data followed a rectangular hyperbola binding isotherm (*23*) and were adequately fit using a single site saturation ligand binding model (R^2 > 0.95 in all cases).

Cesium Desorption from Illite. We selected the IMt-1 illite clay (Cambrian shale, Silver Hill, MT) from The Source Clays Repository of the Clay Minerals Society as a surrogate for soil illite. Characterization of the clay is reported by Hower and Mowatt (*24*). The 0.2−2 μm diameter size fraction was isolated by gravity sedimentation and centrifugation after treatment with $NaC_2H_3O_2$ at pH ∼5 to remove carbonates (*25*). The illite was subsequently treated with KCl to saturate exchange sites with K and was washed several times with deionized water to remove excess salts. The K-saturated illite was oven-dried to a constant mass at 60 °C and stored in an airtight container prior to use. X-ray diffraction patterns did not reveal any impurities in the sample.

Portions of the illite were treated with different concentrations of CsCl to produce a low-Cs illite with approximately

16 mmol kg^{-1} sorbed Cs and a high-Cs illite with approximately 120 mmol kg^{-1} sorbed Cs. Illite was washed several times with deionized water to remove excess salts, and supernatant solutions were analyzed for Cs using ion chromatography (IC). The difference between the measured Cs concentration in the initial solution and the supernatant solutions was assumed to equal the Cs sorbed.

Cesium desorption from illite was determined in suspensions of Cs-treated illite in cell-free bacterial growth media; 0.04 mM sodium citrate ($Na_3C_6H_5O_7$); 0.04 mM sodium oxalate ($Na_2C_2O_4$); or 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions; or in *Bacillus* exudate solutions ranging from 0.1 to 1% in v/v concentration, all in a 0.12 mM NaCl background. All solutions used in these experiments contained equal concentrations of bacterial growth media. The final concentration of cations from growth media solution in each treatment was 0.172 mmol Na, 0.374 mmol NH_4, 0.004 mmol Mg, 0.002 mmol Ca, and 0.02 mmol K. All solutions were maintained at pH 8 throughout the experiments by adding HCl or NaOH dropwise as needed. Preliminary experiments showed that Cs desorption from illite treated with citrate and oxalate solutions reached an approximate plateau after 12−14 days (data not shown). Triplicate samples were mixed on a reciprocal shaker for 16 days, filtered to 0.2 μm, and analyzed for Cs in the filtrate using IC. Additional samples were analyzed for Al using inductively coupled plasma−atomic emission spectrophotometry (ICP−AES).

Measurement of Frayed Edge Site Density. We determined FES concentrations and conditional Vanselow Cs/K selectivity coefficients on the frayed edges of illite ($^{c}K_{ex}^{FES}$) both before and after treatment with experimental solutions using a modification of the method developed by Wauters et al. (*8*). Illite was pretreated with a 100 mM $CaCl_2$/0.5 mM KCl mixed solution, then equilibrated with a 100 mM $CaCl_2$/0.5 mM KCl/0.05 mM CsCl mixed solution for 24 h. The illite was then equilibrated with 100 mM $CaCl_2$ followed by extraction with 1 M NH_4Cl. Frayed edge sites were operationally defined as the sum of Cs and K extracted from the clay by NH_4Cl. Samples were analyzed using ICP−mass spectrometry (ICP−MS) and ICP−AES.

Illite Weathering. We measured Z-averaged hydrodynamic diameters of illite particles in 0.01 M NaCl solution by dynamic light scattering using a ZetaSizer 3000 HAS with a helium−neon laser of 633 nm wavelength (Malvern Instruments, Ltd., Malvern, UK) for illite treated with cell-free growth media; 0.04 mM citrate; 0.04 mM oxalate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions. Illite interlayer spacing was examined on Mg-saturated samples of illite using XRD. Scanning electron microscopy (SEM) was used to examine the surfaces of illite following 240 days treatment with 0.04 mM citrate; 0.04 mM oxalate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions, all at pH 8. This time period approximates the average number of frost-free days annually at the INL site. We used ^{137}Cs isotopic dilution analysis to determine the cation exchange capacity (CEC) of illite following 16 days exposure to 0.04 and 2 mM Na−citrate and Na−oxalate and 240 days exposure to cell-free growth media; 0.04 mM citrate; 0.04 mM oxalate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions, all at pH 8.

Additional samples of K-saturated illite were treated with 0.04 and 2 mM solutions of Na−citrate and Na−oxalate at pH ~8 for 16 days, then were rinsed with deionized water to remove excess salts, air-dried, and crushed lightly. Average diameters of illite particles were measured by dynamic light scattering. Magnesium-saturated illite samples were examined for indications of interlayer hydration using XRD. We used SEM to examine the surfaces of illite following weathering by 0.04 and 2 mM Na−citrate and Na−oxalate solutions and diffuse reflectance infrared spectroscopy (IR) to examine Al complexation with oxalate at the edges of illite.

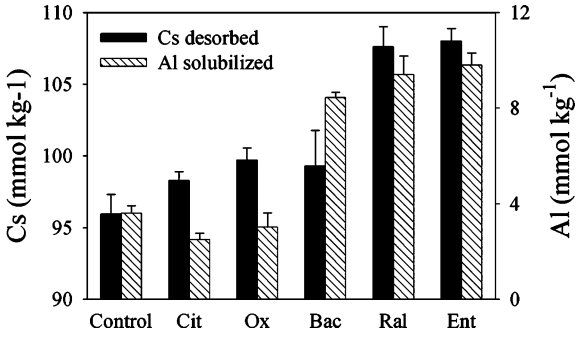

FIGURE 2. Cesium desorption and Al dissolution from illite at pH 8 following treatment with cell-free bacterial growth media (control); 0.04 mM Na-citrate (cit); 0.04 mM Na-oxalate (ox); and 0.2% *Bacillus* (Bac), 1% *Ralstonia* (Ral), and 2% *Enterobacter* (Ent) exudates solutions for 16 days. Initial concentration of Cs on illite was approximately 120 mmol kg^{-1}. Error bars denote one standard deviation.

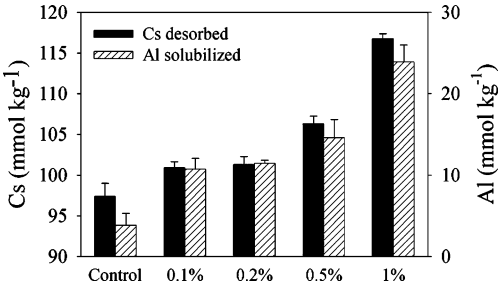

FIGURE 3. Cesium desorption and Al dissolution from illite at pH 8 following treatment with cell-free bacterial growth media (control) and 0.1, 0.2, 0.5, and 1% *Bacillus* exudate solutions for 16 days. Initial Cs concentration was approximately 120 mmol kg^{-1}. Error bars denote one standard deviation.

Statistical Analyses. Single- and multi-factor analyses of variance (ANOVA), paired *t*-tests, and correlation analyses were obtained using SAS 8.0 for Windows (SAS Institute, Inc., Cary, NC). In all cases, statistical significance is reported at the 5% level of significance ($\alpha = 0.05$).

Results and Discussion

Desorption of Cesium in the Presence of Exudates. Bacterial exudates significantly increased Cs desorption from high-Cs illite (120 mmol kg^{-1}) after 16 days (Figure 2). Cesium desorption from illite in the presence of 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions was significantly enhanced as compared to Cs desorption in the cell-free bacterial growth media. Significantly more Cs was also desorbed from illite in the presence of 1% *Ralstonia* and 2% *Enterobacter* exudate solutions as compared to the 0.2% *Bacillus* exudate solution.

Cesium desorption from high-Cs illite increases as the *Bacillus* exudate concentration increases from 0.1 to 1% in a 16 day treatment (Figure 3). Cesium desorption from illite was significantly enhanced at all *Bacillus* exudate concentrations as compared to the cell-free bacterial growth media. There was no significant difference in Cs desorption between 0.1 and 0.2% *Bacillus* exudate solutions, but significantly more Cs was desorbed from illite in the presence of *Bacillus* exudates at 0.5 and 1% exudate concentrations as compared to the 0.1 and 0.2% exudate concentrations. In addition, we observed significantly more Cs desorption from illite in the presence of the 1% *Bacillus* exudate solution than in the 0.5% solution.

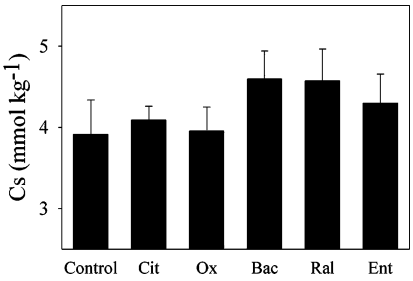

FIGURE 4. Cesium desorption from illite at pH 8 following treatment with cell-free bacterial growth media (control); 0.04 mM Na-citrate (cit); 0.04 mM Na-oxalate (ox); and 0.2% *Bacillus* (Bac), 1% *Ralstonia* (Ral), and 2% *Enterobacter* (Ent) exudate solutions for 16 days. Initial Cs concentration was approximately 16 mmol kg^{-1}. Error bars denote one standard deviation.

TABLE 1. Frayed Edge Site (FES) Concentration on Illite and Vanselow Conditional Cs/K Selectivity of FES ($^cK_{ex}^{FES}$) Following Treatment with Bacterial Exudates[a]

treatment	FES (mmol$_c$ kg^{-1})	$^cK_{ex}^{FES}$ (Cs/K)
control	2.93 ± 0.17	5.74 ± 0.67
0.04 mM citrate	5.03 ± 0.54	3.85 ± 0.39
0.04 mM oxalate	4.13 ± 0.14	5.67 ± 0.60
0.2% *Bacillus*	4.43 ± 0.29	4.16 ± 0.42
1% *Ralstonia*	3.25 ± 0.09	5.48 ± 0.27
2% *Enterobacter*	4.08 ± 0.08	4.48 ± 0.10

[a] Errors denote one standard deviation.

In the case of the low-Cs illite (16 mmol kg^{-1}), only the 0.2% *Bacillus* and 1% *Ralstonia* exudate solutions significantly enhanced Cs desorption after 16 days as compared to the cell-free bacterial growth media (Figure 4). For the low-Cs illite, a greater proportion of Cs was sorbed to highly Cs-selective FES, and the exudate treatments were not as effective in desorbing Cs as in the case of the high-Cs illite.

Several studies have demonstrated that trace quantites of Cs sorb strongly to the highly selective FES of illite (see, i.e., refs *4*−*6*, *8*, *26*−*29*). At higher Cs concentrations, the surface loading on illite increases, and Cs will occupy energetically less favorable regular, or planar, sorption sites. Thus, when illite is treated with Cs, the Cs will sorb first to the most selective sites (the FES), then to the less Cs-selective regular (planar) exchange sites (RES). In the low-Cs illite prepared in these experiments, a large proportion of the total sorbed Cs is sorbed to highly Cs-selective FES and is more difficult to remove than Cs bound to RES. In the high-Cs illite, a larger proportion of the Cs is initially sorbed to RES, which are much less selective for Cs than FES. Cesium is more easily removed from RES than from FES. Consequently, we see greater Cs desorption from the high-Cs illite.

Quantification of FES indicates that the untreated illite exhibits approximately 2.9 mmol$_c$ kg^{-1} due to FES. Assuming that the highly Cs-selective FES preferentially sorb Cs prior to Cs sorption on less selective RES, we can assume that approximately 18% of the Cs sorbed to the low-Cs illite and 2.4% of the Cs sorbed to the high-Cs illite is present on highly Cs-selective FES. It is not surprising, then, that 80−90% of Cs was desorbed from high-Cs illite in the presence of citrate, oxalate, and bacterial exudates as compared to only 25−30% Cs desorption from low-Cs illite.

Frayed Edge Site Densities and $^cK_{ex}^{FES}$ (Cs/K). All examined bacterial exudate solutions significantly increased the density of FES on illite as compared to the cell-free growth media (Table 1). Illite treatment with *Bacillus*, *Ralstonia*, and *Enterobacter* exudate solutions enhanced FES density by 51, 11, and 39%, respectively, as compared to the cell-free bacterial growth media. Citrate and oxalate treatments increased FES by 71 and 41% relative to the cell-free growth media control, respectively. Although FES generally account for only about 1−2% of a soil's total CEC (*26*), the high selectivity of FES for Cs underscores the significance of FES in soils contaminated with trace quantities of radiocesium. Small changes in the quantity of FES in a soil can greatly affect Cs sorption/desorption behavior.

Along with the increase in FES density, in some cases, a concomitant decrease in the Cs/K selectivity of illite FES was observed. The *Bacillus* and *Enterobacter* exudates significantly decreased $^cK_{ex}^{FES}$ (Cs/K) by 28 and 22%, respectively. Citrate treatment decreased $^cK_{ex}^{FES}$ (Cs/K) by 33%. Illite weathering by bacterial exudates and organic acids leads to a decrease in $^cK_{ex}^{FES}$ (Cs/K) with increasing FES density. This result indicates that weathering will increase the biological availability of Cs sorbed to micaceous phyllosilicate minerals because the reduction in exchange selectivity will increase the tendency for Cs to desorb into solution where it can be readily taken up by living organisms.

Aluminum Dissolution from Illite. Both the presence and the concentration of bacterial exudates significantly influenced Al dissolution from illite. Aluminum dissolution was significantly enhanced in the presence of 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions as compared to the cell-free bacterial growth media (Figure 2). Dissolution of Al was also enhanced in the presence of all concentrations of *Bacillus* exudate solutions as compared to cell-free growth media (Figure 3). There was no significant difference in the amount of Al dissolved in the presence of citrate or oxalate and the cell-free growth media.

A significant positive correlation was found between the extent of Al dissolution and the Cs desorption from illite in the presence of citrate, oxalate, and exudates from rhizosphere bacteria in the genera *Bacillus*, *Ralstonia*, and *Enterobacter*. A coefficient of determination of 0.504 was observed between Al dissolution and Cs desorption in the presence of cell-free growth media; 0.04 mM citrate; 0.04 mM oxalate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions. An even greater positive correlation as evidenced by a coefficient of determination of 0.81 was observed between Al dissolution and Cs desorption in the presence of *Bacillus* exudate solutions ranging in concentration from 0.1 to 1%.

Calculations based on Al dissolution indicated less than 1% dissolution of solid illite as a result of exposure to citrate, oxalate, *Bacillus*, *Ralstonia*, or *Enterobacter* solutions. Illite dissolution was calculated from Al dissolution values using the unit cell formula $K_{1.67}[Al_{2.69}Fe_{0.82}Mg_{0.43}Ti_{0.06}][Si_{6.77}Al_{1.23}]O_{20}(OH)_4$ (*24*) to calculate the quantity of Al initially present in the solid illite. Because the initial mass of illite present was known, we were then able to determine the percentage of Al and, consequently, illite that had dissolved.

Although concentrations of bacterial exudate solutions used in these experiments were selected based on Al-binding capabilities and normalized to approximately equal that of 0.04 mM citrate, exudate solutions from *Bacillus*, *Ralstonia*, and *Enterobacter* species all dissolved significantly greater quantities of Al from illite than did the oxalate treatment. This result is similar to that obtained by Kim et al. (*13*), who observed an increase in P dissolution as a result of *Enterobacter agglomerans* growth on hydroxyapatite that could not be accounted for solely by acid production by *E. agglomerans*. Increased solublilization of Al by exudates from *Bacillus*, *Ralstonia*, and *Enterobacter* species in these experiments as compared to the citrate and oxalate solutions indicates that the exudates from the *Bacillus*, *Ralstonia*, and *Enterobacter* species used here are comprised of compounds that exhibit weathering action greater than that shown by citrate or oxalate alone.

These results seem to indicate that the chemical structures of the Al binding components in the supernatants produced by the three bacterial species are different. The activity of Al in the Al binding assay results from a combination of the concentration of the Al binding component and its intrinsic affinity for Al. Solutions of equal Al binding activity, as normalized using the spectrophotometric assay, did not produce equal effects on the cesium-treated illite (see Table 1 and Figures 2 and 6). Since the effect of the *Bacillus* exudate solution increases with increasing concentration (Figure 3), and the three bacterial exudate solutions were tested at significantly different dilutions, we would not expect treatment with equal concentrations of exudate solutions to eliminate the observed differences in the effects of the exudates.

Robert and Berthelin (*14*) suggested that low molecular weight organic acids exuded by living organisms exhibit three types of weathering action: hydrolysis, acid dissolution, and complexation. Acid dissolution is believed to be the primary mechanism of phyllosilicate mineral weathering by organic compounds (*14*). Because of the high stability of Al−citrate and Al−oxalate complexes, both citric and oxalic acid are considered complexing acids (*14*). Exudates of bacteria from the genera *Bacillus*, *Ralstonia*, and *Enterobacter* may exude compounds that weather phyllosilicate minerals through hydrolysis or acid dissolution, as well as those that act via complexation.

Illite Weathering. Some of the microbial products most important to mineral weathering processes are complexing or chelating agents that solubilize elements such as iron, aluminum, copper, zinc, nickel, manganese, calcium, and magnesium from soil minerals (*15*). Microbially derived chelating compounds include simple organic acids (i.e., citric, oxalic, 2-keto-gluconic, tartaric), phenols (i.e., salicylic 2−3 dihydroxybenzoic acids), and trihydroxamic compounds (*15*). Oxalic and citric acids, simple aliphatic acids produced in soil through microbial activity, are effective in forming stable chelate complexes with several metal ions (*15*, *16*, *30*). Citric and oxalic acid treatment leads primarily to mica destruction as opposed to both mica destruction and interlayer expansion (*14*).

Examination of biotite weathering by organic acids has led some researchers to hypothesize that mica minerals weather in two distinct phases (*31*). Boyle et al. (*31*) suggest that H^+ ions first replace interlayer K moving form the mineral edges inward. This renders the interlayer expansible and creates wedge zones formed by the juxtaposition of non-expandible (1.0 nm) and hydrated (1.4 nm) interlayers, or FES. The H^+ ions then move into octahedral and tetrahedral positions, replacing multivalent cations therein and creating a fragile weathered edge (*31*).

Other studies have also shown interlayer expansion, or vermiculitization, of mica as a result of biological weathering processes (*32*−*34*). Hinsinger and Jaillard (*32*) observed in their XRD studies evidence of vermiculitization of phlogopite following K release. Berthelin and Leyval (*10*) reported significantly enhanced biotite weathering by loss of K as a result of corn (*Zea mays*) inoculation with nonsymbiotic rhizosphere microorganisms. In a similar study, enhanced phlogopite vermiculitization and K loss in the rhizosphere of pine (*Pinus sylvestris*) inoculated with *Agrobacterium* sp. was observed following 1−2 years of weathering (*34*). Significant interlayer expansion of biotite mica occurred following soybean (*Glycine max*) growth when inoculated with the mycorrhizae *Glomus macrocarpus* (*33*).

Our XRD analyses of illite did not exhibit any interlayer expansion following treatment with 0.04 and 2 mM citrate and oxalate solutions, nor with 0.2% *Bacillus*, 1% *Ralstonia*, or 2% *Enterobacter* exudate solutions after 16 or 240 days exposure (data not shown). The lack of observed illite interlayer expansion in our samples as compared to the phlogopite and biotite mica may be due to more rapid weathering of phlogopite and biotite as compared to illite. Because phlogopite and biotite are primary trioctahedral minerals, their structures are inherently less stable than illite, which is a secondary dioctahedral mineral.

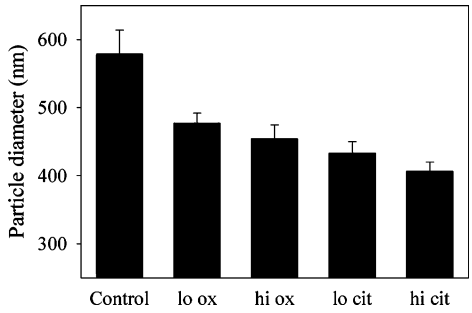

FIGURE 5. Average particle diameter of illite following treatment with 0.04 mM (lo) and 2 mM (hi) Na-oxalate (ox) and Na-citrate (cit) solutions for 16 days. Error bars denote one standard deviation.

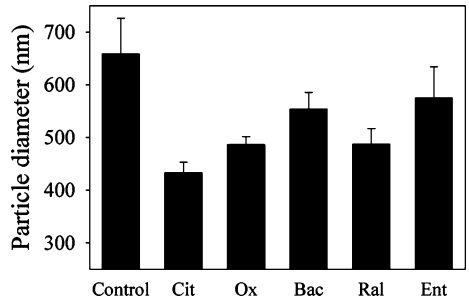

FIGURE 6. Average particle diameter of illite following treatment with cell-free bacterial growth media (control); 0.04 mM Na-citrate (cit); 0.04 mM Na-oxalate (ox); and 0.2% *Bacillus* (Bac), 1% *Ralstonia* (Ral), and 2% *Enterobacter* (Ent) exudate solutions for 240 days. Error bars denote one standard deviation.

We observed a significant decrease in the average particle diameter following treatment with 0.04 and 2 mM citrate and oxalate solutions (Figure 5). In addition, the mean illite particle diameter significantly decreased in the presence of 0.04 mM citrate; 0.04 mM oxalate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions as compared to the cell-free bacterial growth media (Figure 6). More rounded edges were observed on illite treated for 16 days in 2 mM oxalate and citrate solutions than on illite treated in water (Figure 7). Following 240 days exposure to 0.04 mM citrate; 0.04 mM oxalate; or 0.2% *Bacillus*, 1% *Rastonia*, or 2% *Enterobacter* exudate solutions, we observed layer separation and edge peeling in illite samples (Figure 7). We also observed an increased tendency for particle aggregation as a result of illite treatment with Na−oxalate; Na−citrate; or *Bacillus*, *Ralstonia*, or *Enterobacter* exudate solutions. The observed enhanced particle aggregation may be due to edge complexation by organic compounds and bridging effects, binding particles together.

Boyle et al. (*17*) suggested that treatment of biotite mica with organic acids removes cations from the octahedral layer, leaving behind an amorphous matrix. Using IR spectrometry, Varadachari et al. (*35*) found evidence for the formation of a new silica phase on biotite surfaces following treatment with oxalic acid; however, a neoformed silicate phase at the biotite mineral surface was either too small or too sparse to be observed using SEM. Biotite treated with 0.5 M oxalic acid clearly showed fraying of mineral edges in SEM images (*35*). We did not observe any new silicate phases following the treatment of illite by oxalate and citrate or by bacterial exudates; however, observation of illite using SEM following

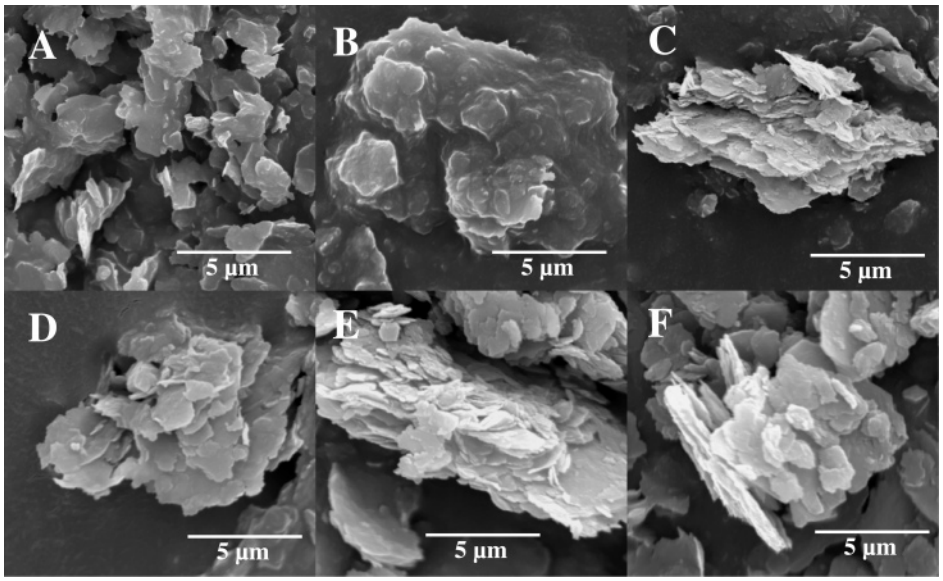

FIGURE 7. Scanning electron microscopy (SEM) images of illite: untreated (A); treated with 2 mM oxalate solution (B) and 2 mM citrate solution (C) for 16 days; treated with 0.2% *Bacillus* exudate solution (D), 1% *Ralstonia* exudate solution (E), and 2% *Enterobacter* solution (F) for 240 days.

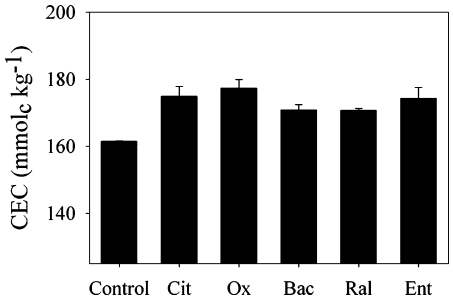

FIGURE 8. Cation exchange capacity (CEC) of illite treated with cell-free growth media (control); 0.04 mM Na-citrate (cit); 0.04 mM Na-oxalate (ox); and 0.2% *Bacillus* (Bac), 1% *Ralstonia* (Ral), and 2% *Enterobacter* (Ent) exudate solutions for 240 days. Error bars denote one standard deviation.

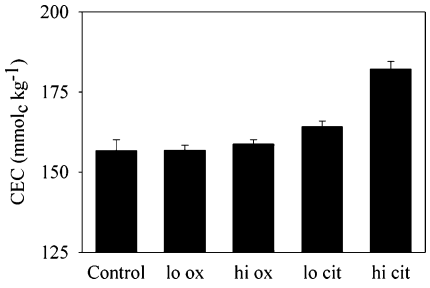

FIGURE 9. Cation exchange capacity (CEC) of illite following treatment with 0.04 mM (lo) and 2 mM (hi) Na-oxalate (ox) and Na-citrate (cit) solutions for 16 days. Error bars denote one standard deviation.

treatment with oxalate and citrate solutions and bacterial exudate solutions provided visual confirmation of illite weathering and alteration of illite edges.

The CEC of illite increased significantly following treatment with 0.04 mM Na−oxalate; 0.04 mM Na−citrate; and 0.2% *Bacillus*, 1% *Ralstonia*, and 2% *Enterobacter* exudate solutions for 240 days (Figure 8). This result is consistent with our results, indicating that FES density increased significantly as a result of treatment with Na−citrate; Na−oxalate; and *Bacillus*, *Ralstonia*, and *Enterobacter* exudate solutions (Table 1). Similarly, the CEC of illite increased following a 16 day treatment with 0.04 mM and 2 mM Na−citrate; however, the CEC was not significantly affected by Na−oxalate at either concentration (Figure 9). This result is surprising since both 0.04 mM oxalate and 0.04 mM citrate enhance the formation of FES on illite (Table 1), and SEM images showed enhanced illite weathering by both the 0.04 and the 2 mM oxalate and citrate solutions (Figure 7). It may be that CEC is increased by separation of illite layers resulting in a smaller number of illite layers per particle. This would expose planar sites without necessarily increasing the number of FES.

It is important to consider the effects of the plant rhizosphere on Cs mobility and fate in soil, particularly since radiocesium sources frequently contaminate the surface region (<1 m depth) of vegetated soils (*1*). Results of this study and our previous work (*11*) indicate that exudates from plant roots and rhizosphere bacteria significantly enhance the formation of highly Cs-selective FES on illite. Cesium availability in the rhizosphere of crested wheatgrass is likely to differ from that in bulk soils. Exudates from species in the genera *Bacillus*, *Ralstonia*, and *Enterobacter* significantly increased the magnitude of Cs desorption from illite. The addition of bacterial exudates also significantly increased the concentration of highly Cs-selective FES on illite. Cesium exchange selectivity with respect to K decreased when the illite was treated with bacterial exudate solutions. These results indicate that bacterial exudates enhance weathering at the FES of illite; however, newly formed FES are less Cs-selective, possibly due to increased hydration of original FES as weathering proceeds inward from mineral edges.

The magnitude of illite weathering by exudates from rhizosphere bacteria will be controlled by the composition and concentration of exudates which largely depend on environmental conditions that influence bacterial community size and composition. In addition, the duration of Cs contamination and the location of Cs on illite crystallites will influence the rate of Cs release due to illite weathering. These results strongly suggest that Cs bound to FES of micaceous minerals will become more available to plants and rhizosphere microorganisms that exude Al-chelating compounds. However, we saw no evidence of extensive illite vermiculitization due to weathering by bacterial exudates, indicating that long periods of time may be required to release Cs that has diffused into illite interlayers. Remediation technologies that utilize plants and associated rhizosphere organisms to

release Cs bound to micaceous phyllosilicate minerals are most likely to be successful when applied to sites characterized by relatively recent Cs contamination. The presence of bacteria in the genera *Bacillus*, *Ralstonia*, and *Enterobacter* may enhance Cs bioavailability in the crested wheatgrass rhizosphere.

We initially hypothesized that bacterial exudates with the ability to strongly chelate Al would enhance mineral weathering by removing Al from mineral structures. For this reason, we selected bacterial exudate concentrations for use in these experiments based on the exudates' ability to complex Al. We conducted the experiments described here in an effort to ascertain whether removal of Al from illite by Al-complexing bacterial exudates would enhance the formation of FES and release sorbed Cs. We were particularly interested in the release of Cs from FES and the generation of additional FES on micaceous minerals due to the relatively recent deposition of radioactive Cs at the INL.

Radiocesium has been deposited in surficial (>1 m depth) soils at the INL site as recently as 1995 (*19*). Naturally occurring, nonradioactive Cs has been present in the sediments at the INL since they were deposited. The distribution and bioavailability of this Cs in the soil is the result of thousands of years of weathering of Cs-containing minerals. Studies have demonstrated that Cs sorption to illite is characterized by initially rapid uptake followed by slower diffusion processes and is significantly affected by the nature of competing cations in solution (*4*−*6*, *26*−*29*). The initial rapid Cs sorption is believed to occur on highly selective FES followed by slow diffusion into energetically favorable interlayer sites. The rate of Cs movement into the clay interlayer is dependent on the competing cations, and the fraction of sorbed Cs occupying interlayer sites is often considered to be nonexchangeable. This suggests that the recently deposited radiocesium is likely to be found primarily on the more exchangeable FES, whereas the nonradioactive Cs is predominantly bound in the nonexchangeable interlayer sites. Thus, the generation of additional FES and removal of sorbed Cs from highly selective FES by exudates from bacteria isolated from the rhizosphere is likely to be a significant process affecting the biological availability of radiocesium in INL soils.

Acknowledgments

The authors thank Eric King for isolation of bacterial strains and preparation of bacterial exudates, Dr. William Keener for sequencing 16S rDNA of bacterial strains, and Dr. Youjun Deng for assistance obtaining SEM images. This work was supported by the National Science Foundation Integrated Graduate Education and Research Training (IGERT) Program under Grant NSF DGE-9972817 to Washington State University and by the Inland Northwest Research Alliance under Grant WSU 004.

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Received for review July 29, 2004. Revised manuscript received April 11, 2005. Accepted April 14, 2005.

ES048809P